Extraction and Cleanup of XAD-2 Resin Cartridges for Polychlorinated Biphenyls and Trans-Nonachlor

Eric Crecelius and Lisa Lefkovitz
Pacific Northwest National Laboratory
Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, WA 98382

Standard Operating Procedure MSL-M-091-00

June 1994

Extraction and Cleanup of XAD-2 Resin Cartridges for Polychlorinated Biphenyls and Trans-Nonachlor

1.0 Scope and Application

This SOP is applicable to the extraction and analysis of polychlorinated biphenyl compounds extracted from fresh water using XAD-2 polymeric resin cartridges. The target compounds which can be determined by this method are, generally speaking, the polychlorinated biphenyls (PCBs) found in Aroclors 1232, 1248, and 1262 and trans-Nonachlor. It may be applied to other compounds once acceptable method recovery has been demonstrated.

Up to 200 liters of filtered freshwater is passed through a glass column containing approximately 400 grams of pre-cleaned XAD-2 resin at a flow rate of approximately 1 liter per minute. This resin has been shown to efficiently extract non-polar organic compounds, including PCBs, from freshwater. The columns are sealed, labeled, and stored under refrigeration until analysis.

At analysis the resin is transferred from the column to an extraction apparatus and rinsed with acetone to remove external water. The surrogate compounds are then added and the resin is then extracted with acetone to remove organics and interstitial water. Next the resin is extracted with a mixed solvent of 50% hexane 50% acetone to remove the remaining organic compounds. The acetone rinse and acetone extract are combined in a separatory funnel, 300 mL of reagent water is added, and the mixture is then extracted once with 200 mL of hexane then twice with 100 mL of hexane to remove PCBs into the hexane extracts. These hexane extracts are then combined with the hexane-acetone extracts, reduced in volume and exchanged into hexane to a final volume of approximately 1 mL. The extract is then applied to a cleanup column which is eluted with 60 mL of hexane. The hexane is reduced in volume to 0.9 mL at which point 0.1 mL of internal standard is added to complete the sample preparation.

Extracts are then subjected to gas chromatographic analysis with electron capture detection (see SOP MSL-093-00). The chromatographic separation is performed using a 60 meter capillary column. Identification and quantitation of the PCB compounds is accomplished by comparison to calibration standards containing a large number of PCB congeners in known concentration.

2.0 Definitions

The following terms and acronyms are associated with this procedure:

DCM	Dichloromethane
GC-ECD	Gas chromatography with electron capture detection
K-D	Kuderna-Danish
NA_2SO_4	Sodium Sulfate

rpm	Revolutions per minute
SRM	Standard reference material

3.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory, 3) defending the data in a Quality Assurance Audit; and 4) reporting results to client.
Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of the analysis; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using this procedure. Responsible for 1) understanding the proper handing of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality assurance samples for each batch of samples analyzed; 4) reporting results to the Project Manager; and 5) participating in QA audits.

Quality Assurance Representative: A qualified staff member assigned to the Quality Assurance Unit. Responsible for monitoring the project activities and conducting Quality Assurance Audits to ensure that 1) analysts have conducted the analysis according to the SOP and that deviations from the SOP have been noted in project files; 2) instrument use and maintenance records are kept correctly; and 3) data have been reported and presented accurately.

4.0 Procedure

4.1 Apparatus and Reagents

4.1.1 Chromatography column 50 mm x 300 mm (Ace Glass Co. #5820-50 or equivalent)

4.1.2 Nylon column end caps with FETFE O-Ring (Ace Glass Co. #5845-50 or equivalent)

4.1.3 Teflon column end cap with 1/4" NPT threaded hole (Ace Glass Co. #5844-78)

4.1.4 Roller apparatus capable of rolling 250 mL Qorpak jars

4.1.5 Separatory funnels (sized to fit sample)

4.1.6 Erlenmeyer flasks, various sizes

4.1.7 Chromatography column, 15 x 250 mm with 250 mL reservoir and No. 2 Teflon stopcock

(Kontes #42080-0222)

4.1.8 Kuderna-Danish (K-D) evaporator apparatus: 250 mL and/or 500 mL reservoir; 3-ball macro Snyder column; 2 or 3 ball micro Snyder column; 10 mL or 25 mL concentrator tube

4.1.9 Hot water bath capable of reaching 100°C, located in a fume hood

4.1.10 Water aspirator vacuum source (Bucchi #B169 or equivalent)

4.1.11 1 Liter Soxhlet extraction apparatus complete with flask and condenser (Ace #6810-10 modified to a 1 L size or equivalent)

4.1.12 1 Liter round-bottom boiling flasks (Ace #6887-53 or equivalent)

4.1.13 Reducing Glass Joint Bushings 34/45 to 24/40 (Ace #5023-21 or equivalent)

4.1.14 Pre-Cleaned XAD-2 Resin, 20-60 mesh, 330 m^2 surface area, 90Å pore diameter

4.1.15 Boiling chips, carborundum, soxhlet extracted or baked at >400° C

4.1.16 Glass wool, soxhlet extracted ≥4 hrs in 50:50 hexane-acetone

4.1.17 Nitrogen evaporation apparatus, N-Evap or equivalent, heated with a water bath maintained at 25-35°C

4.1.18 RapidVap Evaporation System (Labconco)

4.1.19 Glass graduated cylinders

4.1.20 Stainless steel and teflon forceps

4.1.21 Steel rod, 3 mm x 50 cm

4.1.22 Microliter syringes or micropipets

4.1.23 Concentrated sulfuric acid

4.1.24 Solvents – pesticide grade or equivalent

Dichloromethane (DCM)
Hexane
Acetone
Reagent Water (Barnstead Organic-Free or equivalent)

4.1.25 Sodium sulfate – anhydrous, reagent grade, heated to 400°C for ≥4 hr, then cooled to room temperature and stored in a desiccator

4.1.26 Alumina, Sigma F-20 or equivalent, 80-200 mesh

4.1.27 Silica, Amicon Matrix Silica pore diameter 60 Å, particle size 105 μm

4.1.28 Internal standard solution – a hexane solution containing 100 ng/mL of PCB 30, PCB 204, and PCB 103

4.1.29 Surrogate solution – a hexane solution containing 200 ng/mL PCB 14, 50 ng/mL PCB 65, 50 ng/mL PCB 166, and 100 ng/mL dibutyl chlorendate (DBC). PCB numbers refer to their IUPAC designations.

4.1.30 Matrix spiking solution – a hexane solution having a nominal concentration of 1830 ng/mL as total PCBs prepared from the 1994 Aroclor mixture provided by M. Mullins, and 100 ng/mL trans-Nonachlor.

4.1.31 Acid silica gel – 30% (w/w) sulfuric acid

4.2 Sample Handling

Samples shall be kept cold (2-6°C) until analysis. Samples shall be extracted within 12 months of receipt at the lab unless specified otherwise by the Project Manager or project-specific plans. Refer to the project-specific sampling plan for sample collection, preservation, and handling methods.

4.3 Labware Preparation

Prior to use, all glassware, Teflon, and other labware should be washed with hot, soapy water and rinsed with tap water, followed by deionized distilled water. Teflon should be solvent rinsed, teflon stopcocks are sonicated for 30 minutes in dichloromethane. Additionally, glassware must be baked at 450°C for at least 4 hours.

4.4 XAD-2 Resin Preparation

Prior to use, the XAD-2 resin must be solvent extracted sequentially with a number of solvents to remove manufacturing impurities. This process is fully described in Battelle SOP MSL-M-090-00 but is also briefly described here. The resin should be placed in a large soxhlet apparatus (1 liter pot size or bigger) and sequentially extracted for 24 hours with each solvent, first with methanol, then with acetone, hexane, and finally with dichloromethane. Then the resin is sequentially extracted for four hours with each solvent, first with hexane, acetone, and finally with methanol. The methanol is then displaced from the resin by numerous rinses with reagent water and then stored under reagent water in the dark at cool temperatures. This procedure must be followed closely with no shortcuts. At this point the resin is ready to be packed into columns.

4.5 XAD-2 Sampling Column Preparation

A detailed description of how the resin-containing columns used for field sampling are prepared is contained in Battelle SOP MSL-M-090, but a condensed description is also given here. Briefly, the column is fitted with a teflon end cap adapter which has a 1/4" FPT threaded hole through it, to

which PVC vacuum tubing is connected. The tubing is then connected to a water aspirator vacuum source. First, several inches of pre-cleaned glass wool is added and then the column is filled with reagent water. The resin is then added to the column with additional water rinses, and the vacuum source is activated. The resin is not allowed to go dry and more resin is added with water washing until approximately 400 cc of resin has been added to the column. Additional pre-cleaned glass wool is added to the top of the resin, forcing out the water, so as to avoid the

formation of air pockets. More water is added if needed and a nylon end plug is screwed onto the column which displaces water. This is done to avoid creating any air pockets. The column is then inverted and the opposite end is capped in a similar fashion. The columns are then appropriately labeled and stored horizontally in a cool dark place.

4.6 Resin Sample Extraction

4.6.1 A small wad of pre-cleaned glass wool is first added to the soxhlet extractor body to prevent any resin from escaping up the siphon tube exit. Next, a column is opened and the glass wool removed and placed into a soxhlet extractor body. The resin is then transferred, with acetone rinsing, into the same soxhlet body. The last plug of glass wool is then added to the soxhlet and the soxhlet is drained of its contents into a 2 liter separatory funnel. This acetone is saved and put aside until later. The total volume of acetone used for this process is 400 to 800 mL. The smallest volume that effectively transfers the resin is the volume that should be used. The soxhlet body containing the resin is then connected to a 1 liter boiling flask containing several boiling stones, 800 mL of acetone is slowly poured into the soxhlet body onto the resin bed and allowed to siphon into the boiling flask. A 100 μL aliquot of surrogate standard (see Sec. 4.1) is then added to the resin in the soxhlet body and the soxhlet condenser is attached. If the sample is to be spiked, 100 μL of matrix spiking solution is added at this time. Coolant water and the heating mantle are activated and the resin is extracted for approximately 4 hours, the heat is then shut off and the system is allowed to cool to room temperature. During extraction the soxhlet should cycle 4 times per hour. The acetone extraction step is important in removing water from the resin, which is critical in making the resin hydrophobic extractable by the non-polar extraction solvent used in the next step.

4.6.2 The soxhlet apparatus is removed from the condenser and tilted to force the acetone extract to siphon into the 1 liter boiling flask. This extract is then combined with the rinse acetone in the 2 liter separatory funnel. *Note: To avoid contamination of the sample from material on the exterior of the neck, before transferring extracts from containers with ground glass joints always rinse the exterior of the necks with the same solvent as that being transferred.* At this point fresh boiling stones and 800-850 mL of 50:50 hexane-acetone are added to the boiling flask, the flask is connected to the soxhlet body, the condenser is connected and the cooling water and heating mantle is activated. The resin is extracted with this solvent system for $\geq$16 hrs after which the heat is then shut off and the system is allowed to cool to room temperature. During the extraction the soxhlet should cycle 4 times per hour. After extraction the soxhlet body is tilted to cause the solvent to be siphoned over into the boiling flask, where it will be evaporated.

4.7 Acetone Extract Back Extraction

To accomplish this step 300 mL of reagent water is added to the combined acetone rinse and extract contained in the separatory funnel and this mixture is then extracted once with 200 mL hexane and twice more with 100 mL hexane. This step is performed in order to reduce the amount of polar or water soluble interferences being carried through the procedure and to remove water from the acetone prior to evaporation. The hexane extracts are collected in a 1 liter boiling flask for evaporation. If emulsions are encountered or the acetone:water and hexane phases do not cleanly seperate, additional reagent water and/or a small amount of baked sodium sulfate may be

added to facilitate separation of the phases.

4.8 Extract Evaporation

Several fresh boiling stones are added to the hexane-acetone extract contained in the boiling flask, a 24/40 3-ball macro Snyder column is connected to the flask by means of a reducing bushing, the Snyder column is wetted with hexane, and the extract is evaporated to approximately 300 mL. This extract is then quantitatively transferred with several hexane rinses (see note in Sec. 4.6.2) to the boiling flask containing the hexane back-extract of the acetone rinse-extract. Several fresh boiling stones are added, a 3-ball macro Snyder column is attached and wetted, and the solvent is evaporated to a volume of approximately 200 mL. The contents are then transferred to a 250 mL K-D apparatus, fresh boiling stones are added, and the extract is reduced to an apparent volume of 1-5 mL. The K-D is allowed to cool and the 250 mL flask and macro Snyder column are removed from the concentrator tube. Fresh boiling stones are added, a micro Snyder column is attached and wetted with hexane, and the volume is reduced to approximately 1 mL. To effect a complete exchange into hexane fresh boiling stones and 10 mL of hexane are added and the volume is again reduced to 1 mL. This final step is then repeated once more and the volume brought to approximately 1 mL. Any evaporation device (such as the Labconco Rapidvap) that can be demonstrated to yield acceptable spike recoveries, acceptable precision, and acceptable levels of contamination can be used in place of the K-D apparatus.

4.9 Column Chromatography Clean-up of Extract

A silica/alumina cleanup column is used to remove polar interfering compounds remaining in the extract prior to GC analysis.

4.9.1 Prepare 10% deactivated Alumina and 6% deactivated silica by activating a portion of each by heating to 400°C for at least 4 hours and allowing to cool to room temperature in a desiccator. Weigh a portion of the alumina or the silica into a glass jar with TFE-lined lid. Add a weight of water equal to the percent deactivation desired (either 10% or 6%) based upon the weight of he portion used. Place jar on roller for 30 minutes. Store in a sealed glass container. The deactivated material must be used within 24 hours of preparation or this procedure must be repeated. After initial heating to 400°C both the alumina silica gel must be stored either at approximately 130°C or in a desiccator prior to deactivation.

4.9.2 Prepare acid silica gel (40% w/w) by thoroughly mixing the appropriate portions of concentrated sulfuric acid and activated silica gel together in a clean container. The amount of concentrated sulfuric acid to be used for any weight of activated silica gel can be calculated by using the following equation:

(0.36) X (gms Silica) = mLs conc. H_2SO_4.

Break up aggregates with a stirring rod or place the container on a roller table until a uniform mixture is obtained. Store in a glass jar with a TFE-lined lid.

4.9.3 Prepare a column by placing a small portion of glass wool at the bottom of a chromatography column. Pour 70 mL hexane into the column filling it to a level that is

approximately 1/3 the volume of the reservoir. Place a powder funnel in the column and pour 10 g of the 10% deactivated alumina into a column, swirling the hexane and the alumina allowing the alumina time to completely settle. Add 3 g of 6% deactivated silica to the column in the same manner. Before adding the sodium sulfate open the stopcock and allow the hexane to slowly drain, then add enough sodium sulfate to result in a plug approximately 1 cm high. The acid silica gel must be slurry packed in order to prevent trapping bubbles in the column. Into a small beaker containing 8 g of acid silica gel, pour a sufficient amount of hexane to immerse the adsorbent. Swirl the beaker to release most of the bubbles, then with the aid of a squeeze bottle of hexane, pour the slurry into the column, swirling it to aid settling. Again using the same technique as described above, add enough sodium sulfate to result in a plug approximately 1 cm high. Drain the solvent level down to just above the top of the sodium sulfate. Place a 250 mL K-D apparatus under the column to collect the eluent.

4.9.4 The 1 mL sample extract from 4.8.1 is carefully transferred to the column with several small hexane rinses. A small portion of hexane should be used to rinse down the sides of the column. Allow each rinse to drain to just above the sodium sulfate layer. Carefully add a *total* of 60 mL of hexane to the column (including rinses) and allow the column to drain completely, collecting all the eluent.

4.9.5 Add boiling stones to the K-D containing the eluent and reduce in volume to 0.9 mL (as measured in the 10 mL concentrator tube) using macro and micro Snyder columns followed by nitrogen evaporation using a stream of ultra high purity nitrogen. At this point add 100 μL of internal standard solution to bring the volume to 1.0 mL and transfer to an autosampler vial for analysis.

4.10 Quality Control Sample Frequency

Samples prepared using this procedure should be processed in batches sized in accordance with the project analytical QAPjP. The QA/QC samples described below and their frequency are guidelines; the project specific QAPjP should be consulted prior to beginning any analysis of sample preparation. Additional QA/QC samples may be required as specified in the project specific QAPjP.

4.10.1 Lab Procedural Blank – prepare one per batch. Prepared by working through the sample preparation procedure using only solvents and reagents.

4.10.2 Lab Matrix Blank – prepare one per batch. This is a non-field exposed resin sample prepared in a manner identical to that used for field samples.

4.10.3 Spiked Matrix Blank – prepare one per batch. This is a lab matrix blank fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.4 Spiked Procedural Blank – prepare one per batch. This is a lab procedural blank which is fortified with target analytes and prepared in a manner identical to that used for field samples.

4.10.5 Sample Replicates – analyze one sample per batch in duplicate.

4.11 Data Recording and Storage

All standard preparation data will be recorded in accordance with SOP MSL-M-056.

All extraction data and sample extraction information will be recorded on the XAD-2 Resin and Filter Extraction Data Sheet (Attachment 1).

All transfers of data to forms and data reductions (e.g., concentration calculations, means, standard deviations) will be checked by the analyst and approved by the project manager. Hard copies of GC printouts of calibrations and sample data and spreadsheet reports will be kept in the Chemistry Group Central Files. Analytical electronic data will be archived on magnetic tape.

5.0 Quality Control

Results of quality control samples (e.g., blanks, spikes, intercomparison samples, and replicate samples) prepared using this procedure will meet the criteria given in the project specific QAPjP. Recovery of the surrogates will be used to monitor for extraction efficiency, unusual matrix effects, or sample processing errors. Surrogate recovery criteria will be given in the project specific QAPjP.

Solvents, reagents, glassware, and other sample processing hardware may cause artifacts or interferences to sample analysis. The analyst must demonstrate that these materials are free from interferences under the conditions of the procedure by analyzing method blanks.

6.0 Safety

All analysts following this procedure should be aware of routine laboratory safety concerns, including the following:

6.1 Protective clothing and eyeglasses must be worn at all times when handling samples and chemicals.

6.2 Proper care must be exercised when handling solvents and acids, and when using syringes.

6.3 Extractions of resin samples are only to be performed in the walk-in fume hood located in Room MSL 114 or in the fume hood in Room MSL 231. Both of these hoods are equipped with heating mantles that are equipped with safety earthed ground screens, and with recirculating water chillers that have been equipped with flow sensing devices.

6.4 The purpose of the safety earthed ground screens is to shut off power to the heating mantle unit should the screen (which is located just above the heating element) become electrically connected to the mantle housing, as in the case of a boiling vessel rupturing.

6.5 The flow sensing device on the recirculating chiller will shut off the chiller and the heating mantle unit should coolant water flow be interrupted, as in the case of a hose breaking. This will prevent the boiling flask from boiling dry.

6.6 In addition to these features each hood is equipped with a liquid sensor which, if it detects liquid present in the hood or in the containment trays (used in the walk-in fume hood), will shut down power to both the chiller and the heating mantles.

6.7 If overnight unattended extractions are to be performed the project manager must make arrangements with the Building Director to ensure that at least once during the off-shifts a security guard checks for any problems in the extraction areas.

7.0 Training Requirements

All staff performing extractions of XAD-2 resin samples for analysis of PCBs and trans-nonachlor compounds must first read this SOP and then demonstrate proficiency in the process prior to performing the work. Proficiency will include demonstrating that 1) a blank having an acceptably low level of contaminants can be produced and 2) that blank spike recoveries are within acceptable recovery range. Documentation of training will be recorded on training assignment and on-the-job training forms from SOP MSL-A-006. Records of this training will be kept by the laboratory Quality Assurance Representative.

8.0 References

J. I. Gomez-Bellinchon, Grimalt, J. O., and Albaiges, J., "Intercomparison Study of Liquid-Liquid Extraction and Adsorption on Polyurethane and Amberlite XAD-2 for the Analysis of Hydrocarbons, Polychlorobiphenyls, and Fatty Acides Dissolved in Seawater," Environ. Sci. Technol. 1988, 22, 677-685.

Quality Assurance Plan Green Bay Mass Balance Study "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

Analytical Quality Assurance Project Plan (QAPjP) for the EPA Lake Michigan PCB Mass Balance Study, DRAFT, dated October 25, 1994.

ASTM Method D4059-91, "Standard Test Method for Analysis of Polychlorinated Biphenyls in Insulating Liquids by Gas Chromatography."

EPA 660/4-81-045, "The determination of Polychlorinated Biphenyls in TQuality Assurance Plan Green Bay Mass Balance Study" "Cleaning Methods for XAD-2 Resin and Filters" U.S. Environmental Protection Agency (EPA). 1986.

"Analytical Quality Assurance Plan for the Lake Michigan PCB Mass Balance Study."

Attachment 1.

XAD-2 Resin and Filter Extraction Data Sheet, EPA PCB Mass Balance Study

DATE: _____ ANALYST: _____ SOP#: _____

PROJECT/CF#: _____ GLASS WOOL LOT# and MFG _____

CRIUSE: _____ HEXANE LOT# and MFG _____

BATCH#: _____ ACETONE LOT# and MFG _____

RESIN/FILTER: _____

ALUMINA LOT #: _____

SILICA LOT#: _____

Na2SO4 LOT#: _____

SURROGATE STD, no/vol _____

INTERNAL STD, no/vol _____

SPIKE SOLN A, no/vol _____

SPIKE SOLN B, no/vol _____

SAMPLE EXTRACTION					COMMENTS
SAMPLE ID	EXTRACTION DATE	COLUMN BATCH ID	NO. OF FILTERS	WATER VOLUME (L)	